Supporting information

Biological relevance of oxidative debris present in as-prepared graphene oxide

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Table S1. Properties of proteins

Protein	Mol. Wt (kDa)	pI	Net charge (pH 7.0)*	Total number of residues	Number of Arginines	Number of Lysines	PDB ID	Reference
Pepsin A	40	1	-40	326	2	1	3PEP	1
GOx	85	4.6	-27	583	22	15	1GAL	2
HSA	66	4.7	-15	585	24	59	4L9K	3
Ovalbumin	45	4.9	-12	386	15	20	1OVA	4
BLG	18	5.1	-18	162	3	15	1BEB	5
Catalase	64	5.4	-5	527	32	28	3NWL	6
BSA	66	5.5	-17	583	23	59	3V03	7
Hb	64	6.8	1	572	14	48	2QSS	8
Mb	17	6.8	0	153	2	19	3WI8	9
RNase A	14	9.3	4	124	4	10	4POU	10
Trypsin	24	9.3	7	281	2	14	1TPA	11
Cyt *c*	12	10	13	104	2	16	3CYT	12
Lyz	15	11.3	8	129	11	6	1LYZ	13

*Theoretical – from known sequence.

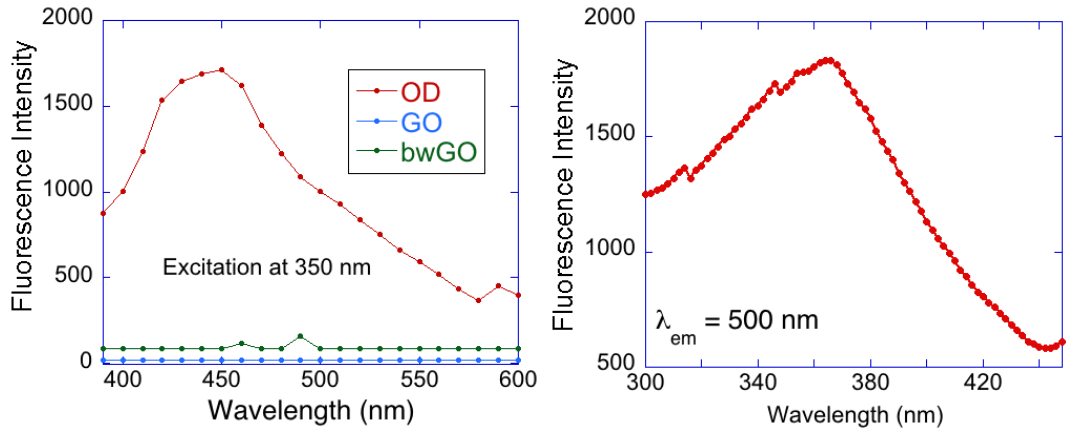

Figure S1. A. Fluorescence spectra of OD (50 µg/mL), GO (150 µg/mL) and bwGO (150 µg/mL) in 10 mM sodium phosphate buffer at pH 7.0. OD shows a characteristic peak at 450 nm (excitation at 350 nm) whereas GO and bwGO showed no noticeable peaks at the same region, proves the separation step. B. Excitation spectra of OD (200 µg/mL) show emission maxima around 350 nm, with emission monitored at 500 nm.

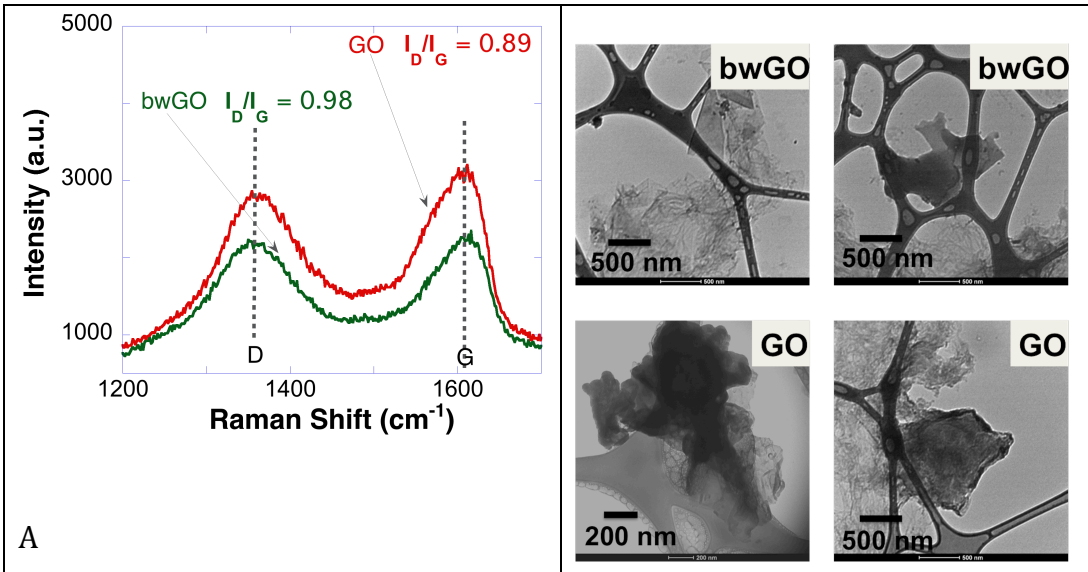

Figure S2. A. Raman spectra of GO and bwGO shows no significant change in position or intensity of D and G bands This proves that no additional defects were introduced to the graphitic plane by base wash. B. TEM images of GO and bwGO show similar morphology.

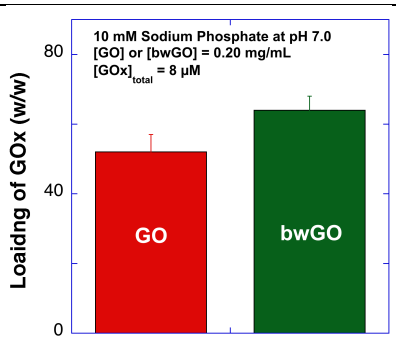

Figure S3. Loading efficiency (%) of GOx/bwGO (red) and GOx/bwGO (green) shows increased binding of GOx to bwGO.

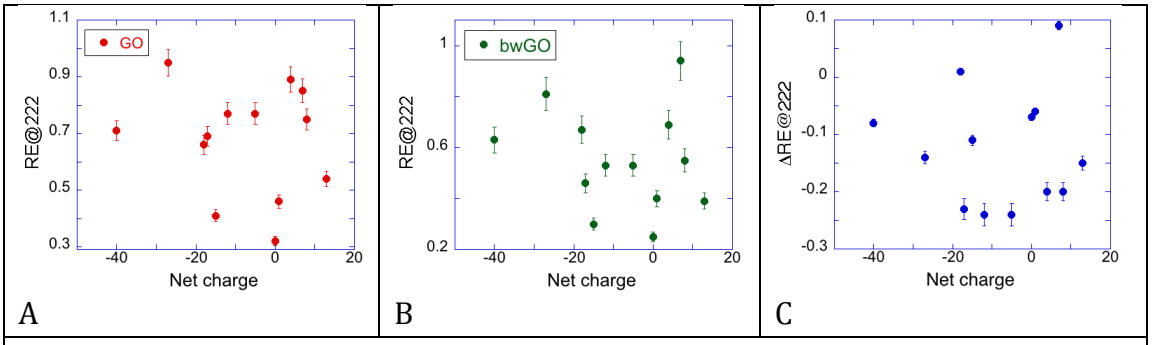

Figure S4. Net charge of the protein at pH 7.0 is not correlated with relative loss of ellipticity at 222 nm (RE@222), after binding to GO (A) and bwGO (B). The differences in ellipticity retention of bwGO to GO (ΔRE@222) is shown in C. No noticeable trend is shown in all cases which indicates that the role of charge in structure retention of proteins is negligible.

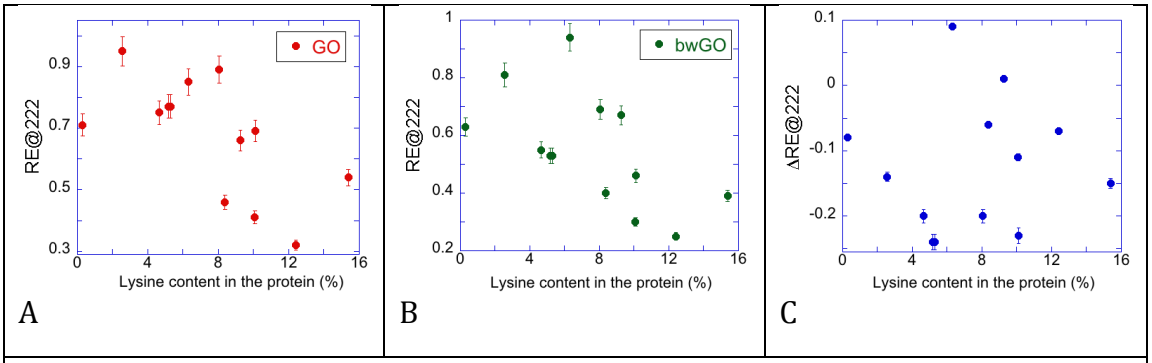

Figure S5. Plots of lysine content in the protein (%) vs the extent of structure retention observed after binding to GO (A) and bwGO (B). (C) The difference in the extents of structure retention (ΔRE@222) as a function of lysines. Lack of correlation in each case was noted.

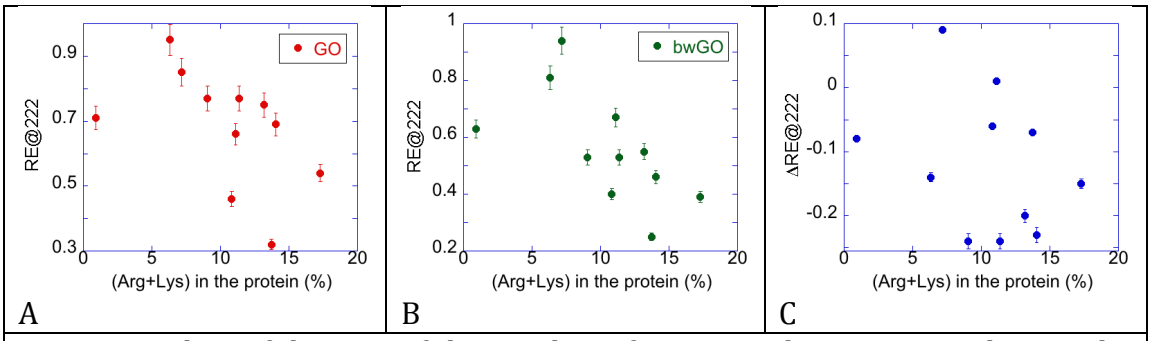

Figure S6. Plots of the sum of the number of Lysine and arginine residues in the protein as a function of the extent of structure retention after binding to GO (A) and bwGO (B). (C) The difference in the extents of structure retention (ΔRE@222) for bwGO/protein minus GO/protein as a function of total number of arginines and lysines combined, and the absence of any correlation is demonstrated.

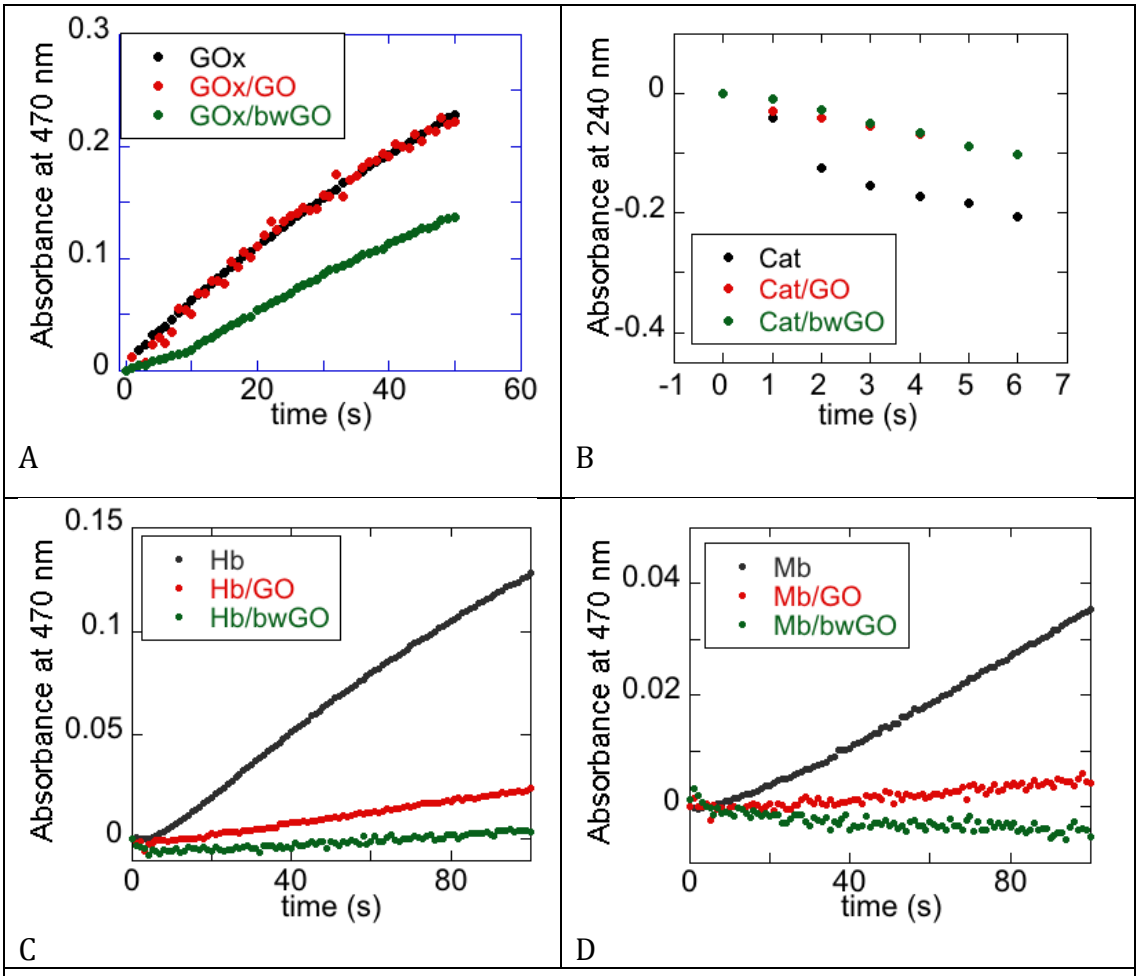

Figure S7. Activity traces of oxidase activity of GOx (A), peroxide reduction activity of Cat (B) and peroxidase like activities of Hb (C) and Mb (D) is shown. The specific activities of free enzymes (black lines), GO conjugates (red dots) and

bwGO conjugates (green dots) were determined from the slope of the lines. Average of three independent measurements is plotted here.

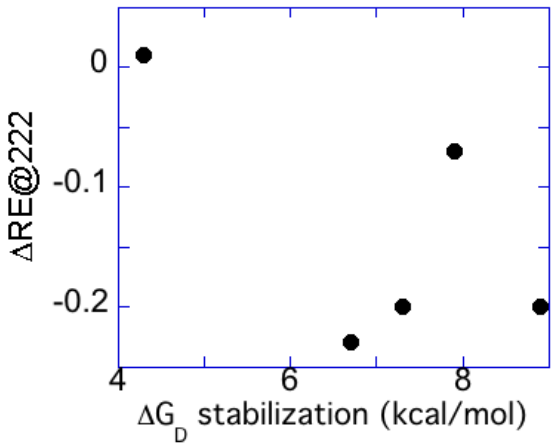

Figure S8. Free energy of denaturation of BLG, BSA, RNase A, Mb, and Lyz[14] (from left to right points in the plot) plotted against ΔRE@222, shows no correlation.

Table S2. Statistical analysis of cytotoxicity data using two-tailed, unpaired student *t* test.

Conc. Of GO/bwGO (μg/mL)	ρ value	Significant (ρ < 0.05)
10	0.5381	No
25	0.5360	No
50	0.8710	No
75	0.4452	No
100	0.0429	**Yes**
250	0.0176	**Yes**
500	0.0237	**Yes**

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